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13. ABSTRACT *(Maximum 200 Words)*

Recently, advances in tumor immunology and identification of tumor-associated antigens (TAAs) have led to an increased interest in vaccination strategies to treat cancer and/or prevent cancer relapse. Immunotherapy can be used as an adjuvant with other systemic therapy to target micro-metastatic disease and prevent cancer recurrence. Although preclinical/clinical trials using cloned tumor-associated antigens for tumor vaccination are ongoing in melanoma and other cancers, breast cancer immunotherapy is limited due in part to the limited number of breast tumor-associated antigens identified to date. Using serological identification of antigens by recombinant expression cloning (SEREX), we first identified novel tumor antigens in a mouse model of adenocarcinoma. These studies were extended to human breast cancer, and candidate human breast tumor antigens were identified using patient sera. The antigens were characterized at the molecular level with regard to sequence, expression pattern and immunoreactivity with a panel of cancer patient sera. These antigens may serve as potential targets for breast cancer immunotherapy, and may provide insight into breast tumor biology.

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Table of Contents

Introduction

Recent advances in the field of tumor have led to increased interest in vaccination strategies to treat cancer and/or prevent cancer relapse. Animal models suggest that tumor-associated antigens (TAAs), when presented properly by antigen-presenting cells can break host's tolerance toward its tumor and induce specific antitumor immunity, which results in tumor rejection. Immunotherapy can be used as an adjuvant with other systemic therapy to target micro-metastatic disease and prevent cancer recurrence. While the preclinical/clinical trials focusing on using identified tumor-associated antigen for tumor vaccination are ongoing in melanoma and other cancers, breast cancer immunotherapy is limited, in part due to the limited numbers of breast tumor-associated antigens identified to date. Here, we used a serological approach to identify candidate breast tumor associated antigens and to characterize these antigens at the molecular level. Initial work focused on validating this approach (serological identification of antigens by recombinant expression cloning, SEREX) in a mouse model of adenocarcinoma. Two tumor antigens were identified. The studies were next extended to human breast cancer, using cDNA libraries derived from primary breast tumor and breast cancer cell lines. Candidate human breast tumor antigens have been identified, isolated, and characterized at the molecular level. Studies in the final year have focused on investigating the potential for these antigens to act as targets for immunotherapy.

Statement of Work (Revised 11/99)

Aim #1:

Months 1-12

1. Obtain tissue and sera from UAB's tissue procurement facility. Create cDNA library from breast cancer cells and screen with patient sera **(completed)**

Aim #2:

Months 6-24

1. Plaque purify positive clones, and sequence cDNA insert **(completed)**
2. Analyze sequence and compare to databases **(completed)**
3. Perform Northern blot analysis to study RNA expression pattern in normal and malignant tissue **(completed)**

Aim#3

Months 18-36

1. Transfect carcinoma cell clones with phagemids encoding the TAA cDNA fragments. Immunize mice with these phagemids and subsequently challenge with the stably transfected carcinoma cell clones in order to test for antitumor effects of PNV *(this task was deleted in modified statement of work, 11/99)*

Modified statement of work (11/99)

Task 1: Complete molecular characterization of candidate tumor antigens:

complete sequence analysis of tumor antigens (specifically clone 2-1-1) **(completed)**
complete expression analysis by Northern blot in tumor and normal tissue **(completed)**
complete studies of reactivity of antigens with allogeneic patient sera **(partially complete)**
complete mutational analysis **(completed)**

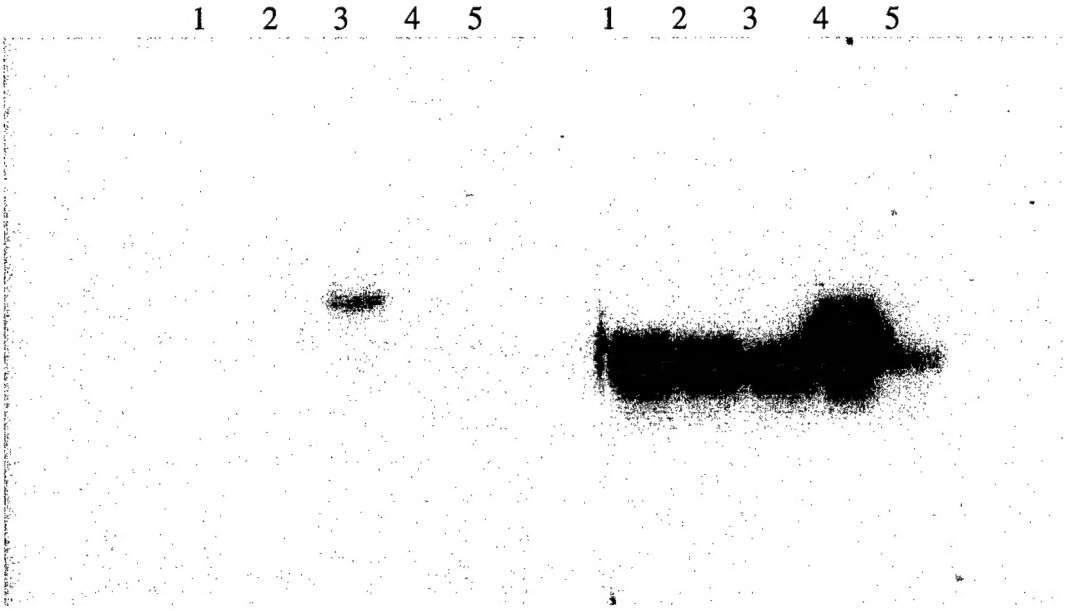
Task 2 Assess immunogenicity of putative tumor antigens: **(partially complete)**

produce RNA
transfect dendritic cells and assay for ability to induce primary immune response in autologous T cells in healthy individuals and individuals with breast cancer

BODY

Work in this final year of funding has focused on completing the molecular characterization of candidate breast tumor antigens. The studies have focused on two antigens described in the previous annual report, S3-2 and 2-1-1.

Expression of S3-2: Our previous studies had shown that S3-2 is highly expressed in breast cancer cell lines and tumors. Sequence analysis of the cDNA insert revealed that it had portions that appear to be derived from two different genes. Further sequence analysis and analysis of genomic databases revealed that these separate genes (NADH oxidoreductase, subunitB14.5b and MGC:2376, hypothetical protein) actually reside on the same chromosome and are most likely adjacent. To determine if the genes are expressed as a single transcript, the cDNA insert was amplified in two pieces and recloned in separate pieces, separating the two putative genes. The cDNAs were then used separately on duplicate Nothern blots. The results suggest that it is actually the NADH oxidoreductase transcript that is highly expressed in breast cancer cell lines and tumors:



MGC:2376, hypothetical protein NADH oxidoreductase B, 14.5b

Northern blot analysis of total RNA derived from cell lines and normal tissue. Previous studies using human breast tumors and cell lines had shown high levels of expression using the S3-2 insert as a probe. Expression in normal human tissues was low. Here, the probe was divided into two probes, one encoding the MGC:2376 portion, and one encoding the NADH oxidoreductast B14.5b subunit. Each probe was radioactively labeled and hybridized to replicate blots. Only the NADH oxidoreductase probe showed high level expression in all tumor cell lines evaluated, but not normal human tissue, as had been seen using the S3-2 probe. Thus, the NADH oxidoreductase gene is highly expressed in breast cancer cell lines and tumors. The transcript is approximately 1 kb in size. (Lanes M=marker, 1. MCF-7, 2. MDA-MB-453, 3. -MDA-MB231, 4. Mel888, 5. Normal ovarian tissue (control normal tissue).

M-phase phosphoprotein 1(MPP-1) Our previous studies suggested the potential utility of clone #2-1-1, which encodes a portion of the M-phase phosphoprotein-1, as a tumor antigen, since its expression was very low in normal tissue and it is highly expressed in tumor tissues. However, a new report of autoantibodies to MPP1 associated with idiopathic ataxia (Fritzler et al, J Invest Med 48:28-39, 2000) raised concerns as to the suitability of this candidate antigen for immunotherapy. Since the cDNA clone described by Fritzler et al overlapped with the cDNA clone we had isolated, we first evaluated whether the region of overlap contained the epitope recognized by the breast cancer patient sera. The overlap region and non-overlap region were cloned into a protein expression vector (Topo-His tag protein expression system, InVitrogen). Expression of the proteins and western blot analysis suggests that the

epitope recognized by the breast cancer patient antibodies overlaps with the region of protein implicated as the antigenic epitope in patients with autoantibodies to MPP-1 and ataxia. Thus, this protein would not be a candidate for immunotherapy approaches to breast cancer treatment. In collaboration with Dr. Peter King, a neurologist with expertise in paraneoplastic syndromes at the University of Alabama at Birmingham, we have obtained serum from cancer patients with paraneoplastic syndromes to determine if serum from these patients contains antibodies to MPP-1. This work is ongoing.

Key Research Accomplishments

1. SEREX was validated in a mouse model of adenocarcinoma: Two tumor antigens have been identified and characterized at the molecular level (Hampton et al., Cancer Gene Ther 7:446-455, 2000 – reprint submitted in the 2000 annual report).

2. Polynucleotide immunization studies indicate that tolerance to these two antigens can be broken using this approach; however, antitumor immunity was not achieved using this vaccine strategy. This suggests that a modified strategy will be needed for effective antitumor immunization in breast cancer patients.

3. Outside the scope of this grant, studies of one of the tumor antigens identified in the mouse model (by Hampton et al) were extended to human breast cancer. The mentor on this grant (T.V. Strong), along with other investigators, described expression of the envelope gene of the human endogenous retrovirus in human breast cancer (Wang-Johanning, F, AR Frost, GL Johanning, MB Khazaeli, AF LoBuglio, DR Shaw and TV Strong. *Expression of Human Endogenous Retrovirus K Envelope Transcripts in Human Breast Cancer.* Clinical Cancer Res, 7:1553-1560, 2001.) Dr. Wang-Johanning also received funded from the American Cancer Society to continue these studies

4. SEREX screening of a human breast cancer-derived cDNA libraries has identified candidate tumor antigens. The expression pattern of these genes has been determined, the seroreactivity with other breast cancer patients was determined, and mutational analysis revealed no mutations in the expressed sequence. Antibodies to one of the antigens has been found (by others) to be associated with ataxia, an ongoing studies will address whether this antigen may have a role inparaneoplastic syndrome in breast cancer.

Reportable Outcomes

Manuscripts, Abstracts and Presentations

1. Manuscript:

Hampton TA, Conry RM, Khazaeli MB, Shaw DR, Curiel DT, LoBuglio AF, Strong TV. SEREX analysis for tumor antigen identification in a mouse model of adenocarcinoma. Cancer Gene Ther 7:446-455, 2000

2. Abstracts and Presentations:

Hampton TA, Conry RM, Sumerel L, Khazaeli MB, Curiel DT, LoBuglio AF, Strong TV. Serological identification of the murine endogenous leukemia proviral envelope protein as a tumor antigen in MC38 cells. Cancer Gene Therapy 4, 1997.

Strong TV, Guerrero A, Hampton TA, Conry RM, Ruppert JM, Curiel DT, LoBuglio AF. Serological identification of human breast tumor associated antigens by recombinant expression cloning. American Association of Cancer Researchers 39:262, 1998.

Hampton TA, Conry RM, Sumerel L, Khazaeli MB, Curiel DT, LoBuglio AF, Strong TV. The murine endogenous leukemia proviral envelope protein acts as a tumor antigen in MC38 cells. Gordon Research Conference on Cancer Molecular Biology, August, 1998.

Degree Obtained:

The initial graduate student trainee (principal investigator), Tracy Hampton, received her Ph.D. based on this work.

Employment/Training

Tracy Hampton received her Ph.D. and received, as a result of this work, a Postdoctoral Fellowship at Stanford University.

Conclusions

We have found the SEREX approach a viable strategy to identify additional antigens for cancer immunotherapy strategies. Studies in a mouse model led to the identification of endogenous retroviral protein (the envelope protein) as a tumor antigen. These studies were extended outside the scope of this grant, and human endogenous retroviral envelope sequences were identified in human breast cancer. Ongoing studies in this area will explore the potential for these proteins to act as targets for immunotherapy. Additional studies using

the SEREX approach in human breast tumors have identified additional antigenic proteins. One of these, MPP-1 does not appear to be a viable candidate for immunotherapy, since antibodies to this protein have been reported in patients with idiopathic ataxia. Additional studies will be needed to determine if this protein may act as a target antigen in paraneoplastic syndrome which occurs, albeit rarely, in breast cancer patients. Another protein, a subnit of ubiquionone oxidoreductase, is overexpressed in breast cancer cell lines and primary tumors. We intend to continue our studies on the role of this protein in breast cancer biology through additional funding mechanisms.

Personnel receiving pay from this grant:

Tracy Hampton

Alicia Sanders Racelis